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Efficacy of Emodepside plus Toltrazuril Suspension (Procox® Oral Suspension for Dogs) against Prepatent and Patent Infection with *Isospora canis* and *Isospora ohioensis*-Complex in Dogs

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Abstract

Three randomised, blinded and placebo-controlled laboratory studies were conducted to evaluate the efficacy of emodepside plus toltrazuril suspension (Procox® suspension for dogs) against *Isospora canis* and *Isospora ohioensis*-complex. Unweaned puppies were experimentally infected with sporulated oocysts of *I. canis* and/or *I. ohioensis*-complex. In each study, one group was treated during prepatency (2 or 4 days post infection) while dogs in the second group were treated individually after the onset of oocyst excretion of the respective coccidia species. The dogs were treated with the minimum therapeutic dose of 0.45 mg emodepside and 9 mg toltrazuril per kg body weight. Daily faecal oocyst counts from both groups were compared to placebo-treated control groups to determine efficacy.

Dogs treated during prepatent *I. canis* or *I. ohioensis*-complex infection showed significantly lower oocyst counts for up to 12 days compared to the control group. Oocyst counts were reduced by 90.2–100 % while the control groups continued to exhibit an adequate infection, except for one study where efficacy against prepatent *I. canis* infection faded 13 days after treatment. Following treatment of patent *I. canis* or *I. ohioensis*-complex infections, significantly lowered oocyst counts were observed for up to 9 days compared to the control group. Faecal oocyst counts were reduced by 91.5–100 %. In all three studies the number of days with diarrhoea was significantly lower when dogs were treated

Fig. 1 Watery diarrhoea as a result of *Isospora* spp. infection

Fig. 2 Oocysts of *Isospora canis* – scale bar 20 µm. © Bayer

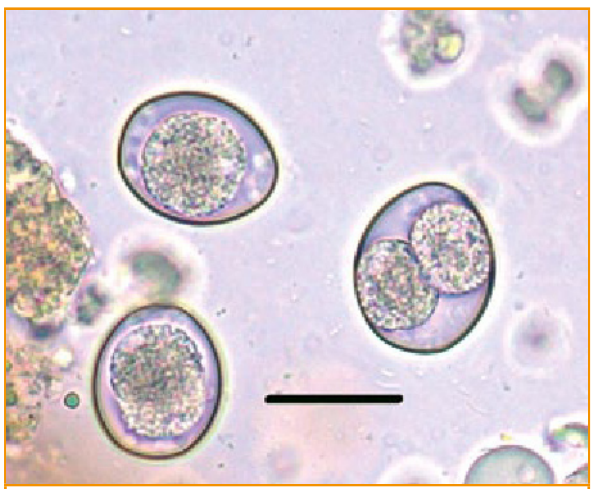

Fig. 3 Oocysts of *Isospora ohioensis*-complex – scale bar 20 µm. © Bayer

during prepatent *Isospora* spp. infection compared to the control groups. No adverse drug reactions were observed during the studies. In conclusion, the studies demonstrated that emodepside plus toltrazuril suspension is an efficient coccidiocide for dogs.

Introduction

Isospora spp. are the causative agents of canine coccidiosis. Clinical coccidiosis mainly affects puppies and immunocompromised dogs with watery, sometimes haemorrhagic diarrhoea (Fig. 1) being the most prominent clinical sign besides vomiting, abdominal discomfort and inappentence (Lappin 2010). Depending on the age of the animal, immune status and the parasite burden, severe dehydration and death can occur (Lappin 2010; Daugschies 2000). While infection often takes a subclinical course, even moderate intestinal damage as a result of *Isospora* spp. infection may considerably alter growth and development of a puppy (Daugschies et al. 2000) so that the significance of *Isospora* spp. infections should not be underestimated. Four *Isospora* species can be found in dogs: *I. canis*, *I. ohioensis*, *I. burrowsi* and *I. neorivolta* (Figs. 2 and 3). The three latter species are often referred to as the *I. ohioensis*-complex because they cannot be reliably differentiated by the size or structure of their oocysts (Lindsay et al. 1997). Although other ways of infection (for example via paratenic hosts) are possible, puppies and young dogs mainly become infected by ingestion of sporulated oocysts from their environment, e.g. from their litter mates with the bitch potentially serving as a reservoir (Daugschies et al. 2000; Buehl et al. 2006). The life cycle of *Isospora* spp. in the dog has been reviewed by Lindsay et al. (1997), Dubey et al. (2009) and others. Following excystation in the gut, cells of the intestinal mucosa are invaded and asexual reproduction (schizogony) takes place. This process is repeated several times before immature oocysts form in the sexual phase of the development cycle

(gamogony). Schizogony and gamogony cause damage to the intestinal mucosa resulting in the above described clinical signs of enteritis. After maturation unsporulated oocysts are excreted in the faeces. The prepatent period after ingestion of infective oocysts is described to be 8–12 days for *I. canis* and 4–9 days for *I. ohioensis*-complex (Becker et al. 1981; Rommel and Zielasko 1981; Baek et al. 1993; Buehl et al. 2006; Mitchell et al. 2007). Sporulation of oocysts passed with the faeces can take place within a few days (Fisher 2002). The sporulated *Isospora* spp. oocysts can then stay infective for months and are frequently resistant to disinfection so that, although basic hygiene measures like disinfection and frequent removal of faeces are recommended, they cannot completely control the contamination of the environment with oocysts (Barutzki et al. 1981; Buehl et al. 2006; Tenter and Deplazes 2006). Therefore, reduction/prevention of oocyst excretion by anticoccidial treatment of dogs is considered to be an essential means to interrupt the life cycle and reduce infection pressure (Daugschies et al. 2000; Buehl et al. 2006).

Emodepside plus toltrazuril suspension (Procox® suspension for dogs) was developed to provide a treatment option for dogs, especially puppies and young dogs, when mixed parasitic infections caused by roundworms and coccidia are suspected or demonstrated. Toltrazuril is a symmetrical triazinetrione and has been used as a coccidiocide in veterinary medicine since the 1980s. It has broad-spectrum activity against species of various genera of coccidia, e.g. *Eimeria* and *Isospora*, and acts against all intracellular developmental stages of schizogony and gamogony by interfering with the respiratory chain and DNA synthesis (Haberkorn and Stoltefuss 1987; Harder and Haberkorn 1989).

The efficacy of the suspension against nematodes (*Toxocara canis*, *Ancylostoma caninum*, *Uncinaria stenocephala*) due to the nematocidal compound emodepside has been demonstrated in a series of laboratory dose-confirmation studies (Schimmel et al. 2011). The present work reports the results of three controlled laboratory studies that evaluated the efficacy of emodepside plus toltrazuril suspension against prepatent and patent infections with *I. canis* and *I. ohioensis*-complex in dogs.

Materials and methods

Study design

The studies were conducted according to good clinical practice as described in VICH guideline 9. Each study contained two treated groups and one control group. The first group was treated 2 or 4 days after experimental infection to investigate efficacy of the formulation against prepatent *Isospora* spp. infection. In the second group, dogs were individually treated one day after the faecal oocyst count (FOC) had reached a defined level, the "FOC threshold" FOC_T [≥ 500 or > 1,000 oocysts per gram faeces (OPG)], to evaluate efficacy of the formulation against patent *Isospora* spp. infections. The control group was treated with placebo at the same time as the first treatment group. The primary variable to determine efficacy were the FOC of treated dogs in comparison to control dogs (see below). The study designs are summarised in Tab. 1.

Study animals

Pregnant, purpose-bred Beagle bitches were obtained from different suppliers so that the puppies to be enrolled in the three studies were born at the test facilities and acclimatised to the study conditions. The puppies were identified by subcutaneously implanted microchips.

Litter mates were housed together with the bitch throughout the studies except for study III, where one litter had to be weaned 7 days post infection (dpi) because the bitch developed clinical signs of coccidiosis that required treatment. In study II three puppies were cross-fostered by one of the bitches to another prior to study start so that litters were of similar size. Puppies received supplemental commercial dog food two or three times daily and water was available *ad libitum*.

General requirements for study inclusion were good health and no recent drug treatment that could potentially interfere with the study.

Clinical observations

In all studies dogs were physically examined at least once before experimental infection and once before treatment. Additionally, all dogs were observed for their general health once daily. On treatment days clinical assessments with the aim to detect adverse events were conducted once before treatment and approximately 0.5, 1, 2, 3, 4 and 8 hours after treatment.

Infection

When the puppies were 3–5 weeks of age, they were orally infected with sporulated oocysts of *I. canis* and/or *I. ohioensis*-complex that had been cultivated at Bayer Animal Health GmbH or the University of Veterinary Medicine Vienna, Austria, originating from puppies of the colony of Bayer Animal Health GmbH or from diagnostic field samples from dogs in Austria. In study I dogs showed a natural *I. ohioensis*-complex infection which could also be included in the efficacy evaluation in addition to the experimental *I. canis* infection. The inoculation doses used for infection are shown in Tab. 1. In study II lower inoculation doses were chosen to avoid serious health complications in the puppies, as experimental infections have been shown to be potentially more pathogenic if *I. canis* and *I. ohioensis*-complex are combined (Becker et al. 1981).

Treatment

Within the litters puppies were randomly assigned to one of the three study groups so that each litter contained treated and control puppies. The puppies were treated once with emodepside plus toltrazuril suspension or placebo suspension. Treatment was applied at set times after experimental infection (2 or 4 days after experimental infection) for groups that were to be treated during prepatency and individually applied the day after FOC_T had been reached for dogs that were to be treated during a patent *Isospora* spp. infection (see Tab. 1).
In all studies the minimum therapeutic dose of 0.45 mg emodepside and 9 mg toltrazuril per kg body weight was administered based on the body weights taken on the day of treatment. The suspension was orally applied using a syringe. The dogs were observed at dosing and ~ 30 minutes after dosing to determine any vomiting or regurgitation.

Tab. 1 Study design

Study no.	Age of dogs (at exp. inf.)	Body weight (day before exp. inf.)	No. of dogs (gr. 1/ gr. 2/ gr. 3)[a]	No. of litters	Infection doses (no. of sporulated oocysts)		Treatment[a] (dpi)	FOC_T	Collection of faeces (dpi)
					I. canis	*I. ohioensis*-complex			
I	4–5 weeks	0.9–2.0 kg	9 / 9 / 9	6	60,000	Natural co-infection	Gr. 1: 4 Gr. 2: 11,12 or 13[b] Gr. 3: 4	> 1,000 OPG	–1, 1, 3, 5 to 18
II	3–4 weeks	0.8–1.4 kg	8 / 7 / 8	4	20,000	11,000	Gr. 1: 2 Gr. 2: 11,12 or 13[b] Gr. 3: 2	≥ 500 OPG	–1, 4 to 20
III	4–5 weeks	0.9–1.8 kg	9 / 8 / 8	5	–	80,000	Gr. 1: 2 Gr. 2: 6,7 or 8[c] Gr. 3: 2	> 1,000 OPG	–1, 1, 3 to 13

Dpi: days post infection; exp. inf.: experimental infection; FOC_T: faecal oocyst count threshold level; OPG: oocysts per gram of faeces
[a] Group (gr.) 1: treatment during prepatency, gr. 2: treatment during patency, gr. 3: control
[b] i.e. day after *I. canis* oocyst excretion had reached FOC_T
[c] i.e. day after *I. ohioensis*-complex oocyst excretion had reached FOC_T

Faecal examination

It was attempted to collect individual faeces from the puppies on the day before the experimental infection and daily at least from 5 dpi until the end of the respective study (for detailed sampling days refer to Tab. 1). Due to the young age of the puppies, it was not possible to obtain faeces each day from each puppy. Individual samples were obtained either by placing the puppies into individual boxes for defaecation or by gentle manipulation of the anal sphincter with a cotton swab to initiate defaecation.

The consistency of faeces was observed directly after collection of faeces. The faeces were assessed as normal/loose (as some variation in faecal consistency was considered to be normal in puppies), diarrhoea or watery diarrhoea. Blood was noted if present. Faeces were stored at room temperature and FOC were conducted within 3 days after collection using modified McMaster techniques.

Efficacy determination and statistical analysis

The primary variable for the evaluation of efficacy against *Isospora* spp. were the FOC of treated dogs in comparison to the control group.

Efficacy was described using two methods:

– % reduction for each day where FOC_T was reached in at least 6 control dogs (> 1,000 OPG in studies I and III; ≥ 500 OPG in study II because of the lower inoculation dose) and
– The days where statistically significant differences ($p < 0.05$) to the control group were seen, independently from specific requirements for infection of the control group.

For the group that received the treatment during patency, the data were analysed relative to the day of treatment instead of the study day, as the dogs were individually treated after passing the respective FOC_T so that within the group dogs had been treated on different study days. Similarly, for the comparison between treated and control group the data in the control group were analysed relative to the day when the individual dogs would have been treated had they been in the treatment group.

Percent efficacy was calculated per study day (group 1) or "day relative to treatment" (group 2) according to the following formula:

% Efficacy (reduction) = (N1 – N2)/N1 × 100

N1: geometric mean FOC of *I. canis* or *I. ohioensis*-complex for the control group (group 3)
N2: geometric mean FOC of *I. canis* or *I. ohioensis*-complex for the treatment group (group 1 or 2).

Geometric means were calculated following transformation using a logarithmic method (averaging the transformed values, and converting the average using anti-log to represent a geometric mean). The non-parametric Wilcoxon rank sum test (two sided, using $\alpha = 0.05$) was used to test for both gender and treatment group effects across all study days.

In study II an additional analysis was performed using a parametric test on ranked oocyst counts. The analysis of the treatment during prepatency involved a repeated measures analysis of variance (RMANOVA) on the rankings. For the analysis of the treatment during patent infection a baseline covariate [average of the day before and the day of treatment log oocyst counts (+1)] was used in a repeated measures analysis of covariance (RMANCOVA). If a statistically significant treatment by day interaction was seen, treatment effects were determined for each study day. If no statistically significant treatment by day interaction was seen, an overall treatment effect was determined for the overall post treatment period.

The analyses were performed using SAS software (SAS® Institute, Cary, NC, USA).

As a secondary criterion, faecal consistency was evaluated by comparing the number of observations of diarrhoea (i.e. diarrhoea ± blood, watery diarrhoea) in each group using the Wilcoxon Rank Sum test (two-sided, using $\alpha = 0.05$). The test results were regarded as significant if the lower bound of the two-sided confidence interval of the Wilcoxon Rank Sum test was > 0.5 (equality line). The results were interpreted only in a descriptive manner because no alpha-adjustment for multivariate testing was performed. The analysis was performed using TESTIMATE Version 6.4 (IDV Gauting).

Results

Experimental infection

In the control groups of studies I and II prepatency after *I. canis* infection was 8–12 days and individual oocyst excretion was observed on 7–11 days with 9 of 17 dogs still shedding oocysts at the end of the studies. During patency, individual oocyst numbers ranged between 0–1 x 10^6 OPG in study I and 0–3.7 x 10^5 OPG in study II. The peak of oocyst shedding was observed 12 dpi (study I) or 13 dpi (study II) with geometric mean oocyst counts of 110,238 OPG (study I) and 18,898 OPG (study II).

In the control groups of studies II and III prepatency after experimental infection with *I. ohioensis*-complex was 3–6 days. Patent infection was demonstrated on 5–17 days with 9 of 16 dogs still showing a patent infection at the end of the studies. During patency, individual oocyst numbers ranged between 0–7 x 10^4 OPG (study II) and 67–1.8 x 10^5 OPG (study III). Peak shedding was observed 6 dpi (study III) or 7 dpi (study II) with geometric mean oocyst counts of 6,437 OPG (study III) and 5,952 OPG (study II). A second, weaker peak was observed 13 dpi in study II with a geometric mean oocyst count of 2,251 OPG.

The intervals where at least 6 dogs in the control group passed FOC_T are shown in Tabs. 2 and 3.

Efficacy evaluation and clinical observations

The efficacy of emodepside plus toltrazuril suspension against prepatent *I. canis* infection was demonstrated in studies I and II (Tab. 2, Figs. 4 and 5). On days when at least 6 control dogs passed FOC_T, oocyst counts were reduced by 90.2–100 % in treated dogs. Significantly lower oocyst counts of the treated dogs were observed for 5–7 days. In study II efficacy was demonstrated for up to 12 days after treatment (14 dpi). For the following three days where the control group still showed at least 6 dogs with counts above FOC_T, no significant differences were seen between the FOC of the treated and control group (Fig. 5).

Treatment with emodepside plus toltrazuril suspension against patent *I. canis* infection reduced FOC by 91.5–100 %, and significantly lowered oocyst counts were observed for 5 to 9 days in treated dogs compared to the control group (Tab. 2). In

Tab. 2 Results of studies on the efficacy against *I. canis*

Treatment	Study no.	Interval of adequate infection[a] in control group	Interval of significant differences between treated and control group	% FOC reduction[b]	
During prepatency	I	12–16 dpi	10–16 dpi	99.8–100 %	(p ≤ 0.010)
	II	11–17 dpi	10–14 dpi	90.2–99.9 % [11–14 dpi]	(p ≤ 0.033)
				no significant reduction [15–17 dpi]	
During patency	I	1–4 dpt	1–5 dpt	96.9–100 %	(p ≤ 0.042)
	II	1–5 dpt	1–9[c] dpt	77.0 % [1 dpt] 91.5–97.6 % [2–5 dpt]	(p = 0.0213)[c]

Dpi: days post infection; dpt: days post treatment
[a] defined as ≥ 6 dogs with oocyst counts reaching FOC_T
[b] % FOC reduction (per day) of treated group during adequate infection of control group
[c] overall treatment effect RMANCOVA

study II efficacy on the day after treatment was 77 % but then was ≥ 91.5 % for the following days.
The efficacy of the suspension against prepatent *Isospora ohioensis*-complex infection could be evaluated in all three studies including study I where a natural *I. ohioensis*-complex infection had arisen during the study. The studies demonstrated FOC reductions between 99.7 % and 100 % and significantly lower oocyst counts for 5–12 days compared to the control group (Tab. 3, Figs. 6–8).
In study III treatment was applied on the day after oocyst counts of *I. ohioensis*-complex had reached FOC_T, while treatment in study II (where animals had been experimentally infected with both *Isospora* spp.) had been targeted at patent *I. canis* infection so that at the time of treatment the dogs had been patent for *I. ohioensis*-complex for some days already. The two studies demonstrated FOC reductions of 99.2 % and 99.9 % and significantly lower oocyst counts for 4–9 days compared to the control group (Tab. 3).
In all three studies the number of days with diarrhoea was significantly reduced when dogs were treated during prepatent *Isospora* spp. infection compared to the control groups (Fig. 9). No differences in the frequency of diarrhoea were seen when dogs were treated only after the *Isospora* spp. infection had become patent.
No adverse drug reactions were observed during the studies.

Discussion

The infections that developed in the control groups following the experimental inoculation of animals with *I. canis* and/or *I. ohioensis*-complex were comparable to published data with regard to length of prepatency/patency and peak of oocyst shedding (Becker et al. 1981; Daugschies et al. 2000; Buehl et al. 2006; Mitchell et al. 2007). The wide ranges for oocyst excretion illustrate the variability typical for coccidial infections and also compare to previous data (Daugschies et al. 2000; Mitchell et al. 2007). *I. canis* generally appeared to lead to higher oocyst excretion than *I. ohioensis*-complex. Similar observations were described by Buehl et al. (2006), who discussed this finding as a possible result of additional schizogony stages of *I. canis*. For *I. canis,* oocyst excretion tended to be higher after a higher inoculation dose, as higher oocyst excretion was observed in study I compared to study II.

Tab. 3 Results of studies on the efficacy against *I. ohioensis-complex*

Treatment	Study no.	Interval of adequate infection[a] in control group	Interval of significant differences to control group	% FOC reduction[b]	
During prepatency	I	6–7 dpi	6–10 dpi	99.7 %	($p \leq 0.025$)
	II	6–8, 12–13 dpi	5–16 dpi	99.8–100 %	($p \leq 0.011$)
	III	5–8 dpi	5–11 dpi	99.9–100 %	($p \leq 0.012$)
During patency	II	1 dpt	1–9[c] dpt	99.2 %	($p = 0.0028$)[c]
	III	1–2 dpt	1–4 dpt	99.9 %	($p = 0.006$)

Dpi: days post infection, dpt: days post treatment
[a] defined as ≥ 6 dogs with oocyst counts reaching FOC_T
[b] % FOC reduction (per day) of treated group during adequate infection of control group
[c] overall treatment effect RMANCOVA

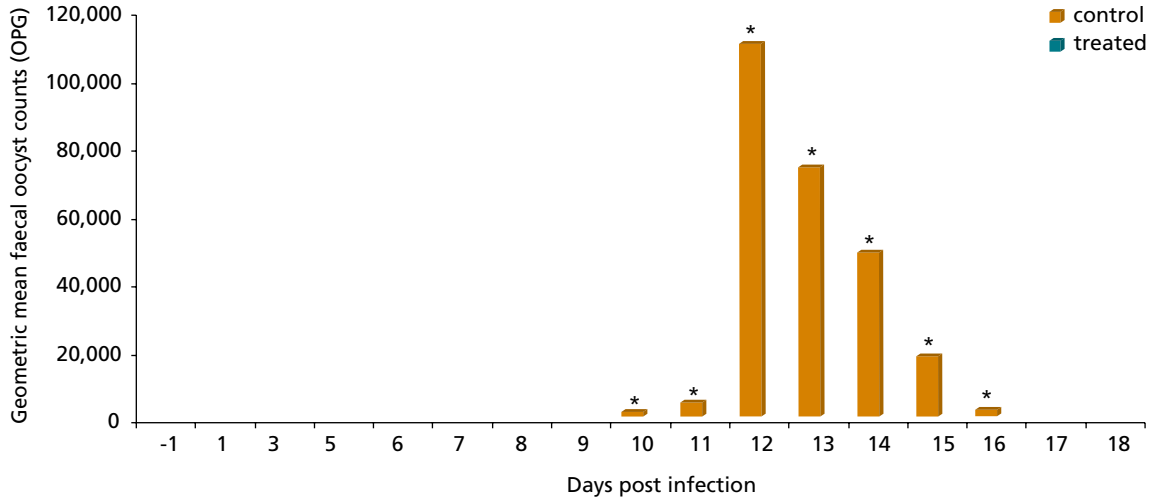

Fig. 4 Study I: Treatment during prepatent *I. canis* infection (treatment 4 dpi, * statistically significant differences)

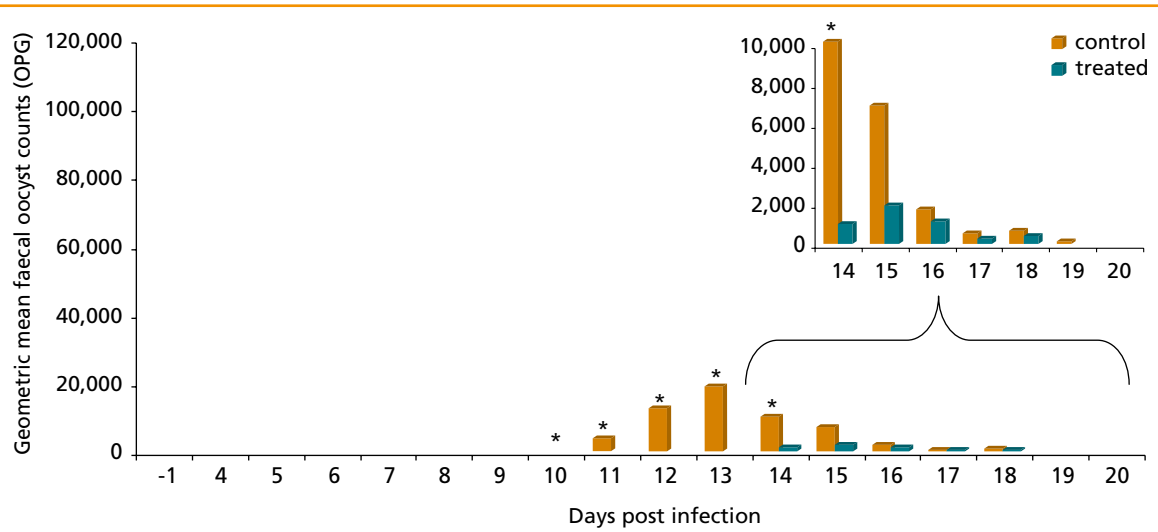

Fig. 5 Study II: Treatment during prepatent *I. canis* infection (treatment 2 dpi, * statistically significant differences)

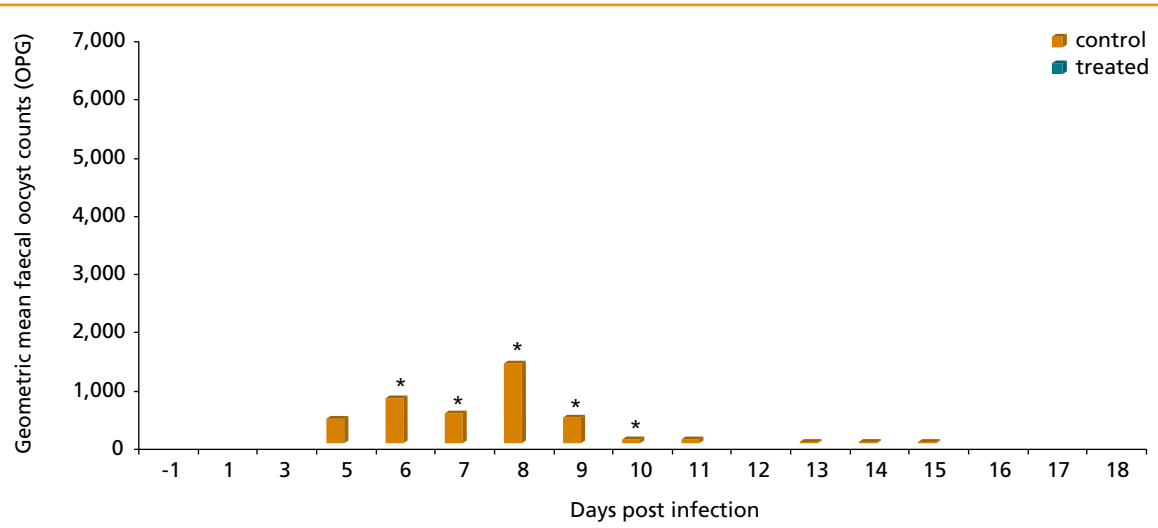

Fig. 6 Study I: Treatment during prepatent *I. ohioensis*-complex infection (natural infection, * statistically significant differences)

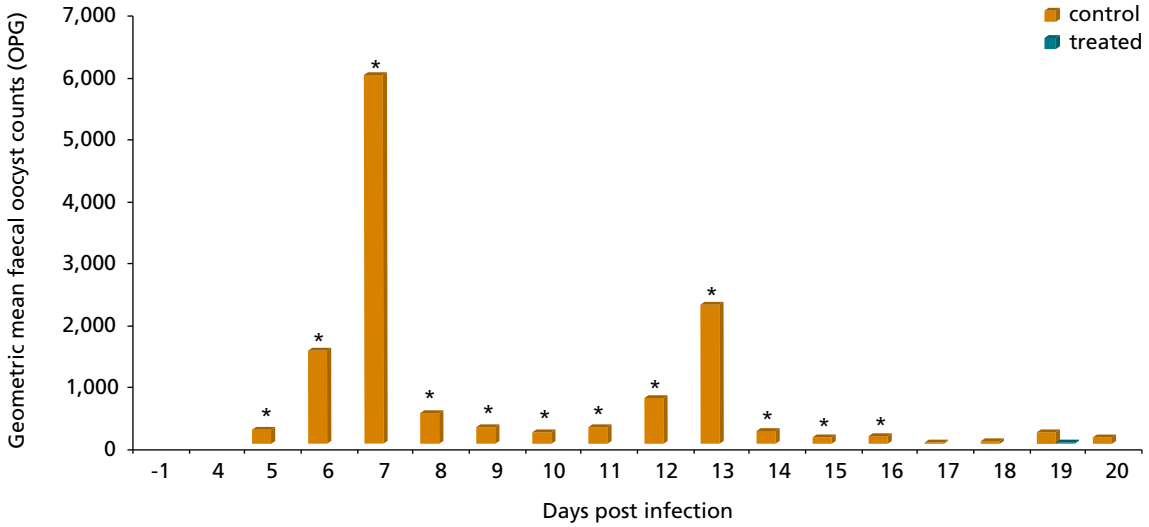

Fig. 7 Study II: Treatment during prepatent *I. ohioensis*-complex infection (treatment 2 dpi, * statistically significant differences)

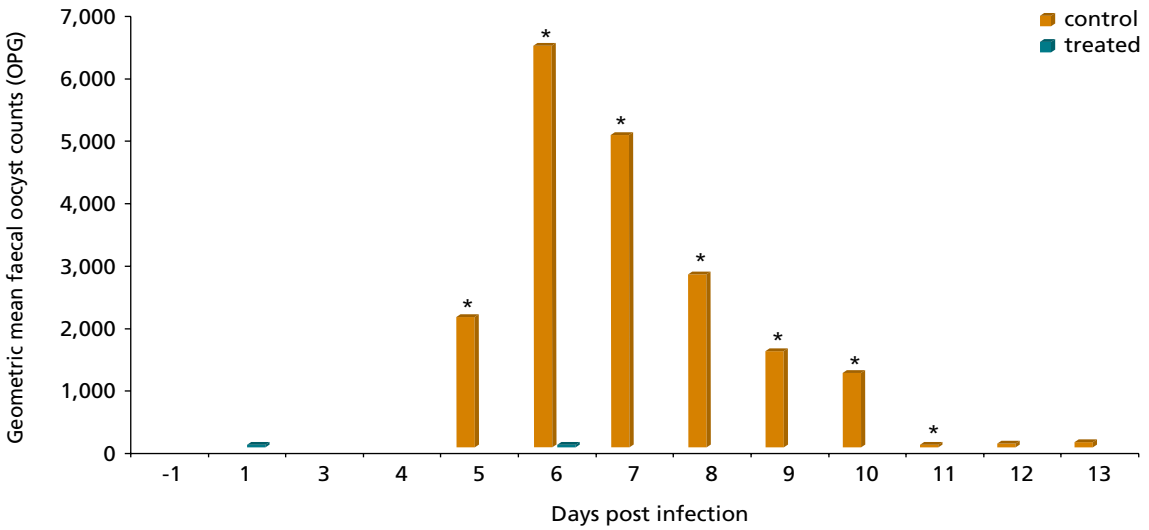

Fig. 8 Study III: Treatment during prepatent *I. ohioensis*-complex infection (treatment 2 dpi, * statistically significant differences)

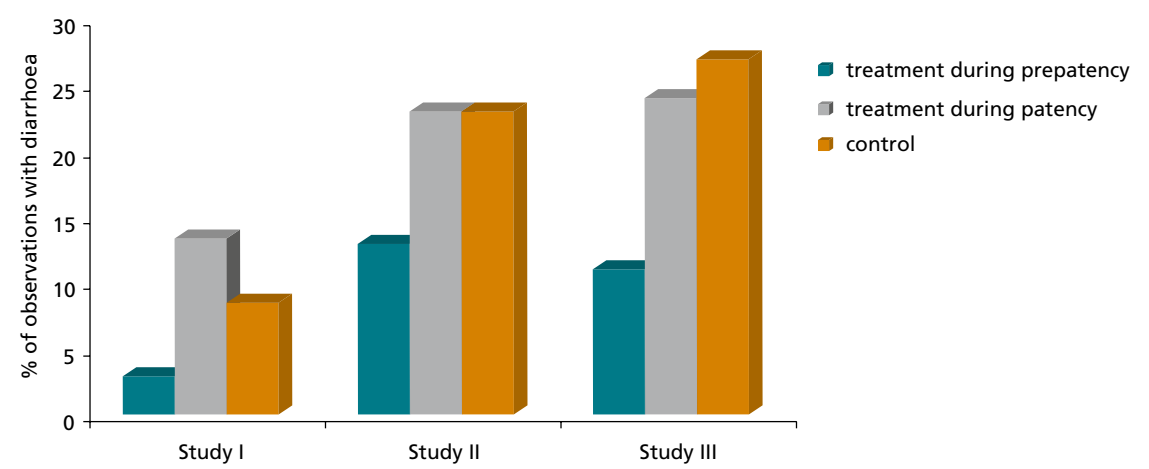

Fig. 9 Results of observations on faecal consistency

In contrast, the differences in the oocyst excretion were less striking between the inoculation doses used for *I. ohioensis*-complex in studies II and III. Interestingly, a second peak was observed 13 dpi for FOC of *I. ohioensis*-complex in study II, where dogs were co-infected with *I. canis*. This peak coincided with the peak for *I. canis* so that the combination of the two pathogens may have altered the excretion pattern of *I. ohioensis*-complex and thus have prolonged patency of *I. ohioensis*-complex. This would differ from the data of Buehl et al. (2006), where no differences had been observed in the excretion patterns of the two species after a mixed infection. High efficacy against prepatent *I. canis* and *I. ohioensis*-complex infection was demonstrated in all three studies. However, in study II there were no significant differences between the *I. canis* oocyst counts of treated and control dogs from 15 to 17 dpi while the requirement for at least 6 control dogs reaching FOC_T was still fulfilled. This could be interpreted as a fading efficacy from 12 days post treatment onwards so that another toltrazuril treatment could be indicated approximately 2 weeks after the first treatment to ensure continued efficacy. However, it should be considered that puppies were randomised within litters, with the result that treated puppies had access to faeces of their untreated littermates. Freshly shed oocysts are not infective so that after ingestion they merely pass through the intestines and cannot be distinguished from oocysts that truly originate from an infection of the sampled individual. This possibility was previously considered by Buehl et al. (2006), who similar to our experimental setup had accepted group housing of litters containing treated and untreated puppies as a potential confounding effect in the interest of animal welfare and sound randomisation practice. Also, study I had yielded very clear results regarding efficacy against *I. canis* with comparatively strong infection of the control group. The metaphylactic efficacy against experimental as well as natural infection with *I. ohioensis*-complex shown in the studies is further supported by a study of Daugschies et al. (2000), where toltrazuril was applied to puppies 3 dpi with *I. ohioensis*-complex. The control dogs showed faecal oocyst counts between 800 and 118,200 OPG while no oocyst excretion was found in dogs treated with 10 mg toltrazuril per kg body weight.

Emodepside plus toltrazuril also showed a high efficacy against patent infections with *I. canis* and/or *I. ohioensis*-complex. The calculated 77 % reduction of *I. canis* FOC in study II on the first day after treatment is considered to be due to the time required for intestinal passage of oocysts. On the first day after treatment, faecal samples can still contain "pre treatment" faeces so that FOC reduction may take a day to become fully evident. This had also previously been observed by the authors in pilot studies (unpublished data).

In addition to the direct effect on FOC, significantly lower occurrence of diarrhoea was observed when dogs were treated during prepatency in comparison to the control group. No such differences were seen when dogs were only treated after the infection had become patent. In *Isospora* spp. infection diarrhoea develops as a result of the enteritis caused by mucosal damage during schizogony and gamogony. The onset of diarrhoea is typically observed shortly before the beginning of oocyst excretion (Mitchell et al. 2007; Lappin 2010). Consequently, if an anticoccidial treatment is applied only after the shedding of oocysts it cannot reverse the damage caused by the endogenous development of the parasites. Depending on the severity of clinical signs additional treatment may be indicated, e.g. fluid therapy against dehydration and possibly antibiotic therapy to prevent or treat secondary bacterial infections that may arise from the intestinal mucosal damage. A certain degree of diarrhoea was still observed in the dogs treated during prepatent *Isospora* spp. infection in the three studies. The puppies were not tested for other potential causes of enteritis like bacteria and viruses so that it is unknown whether such agents may have contributed to occurrence of diarrhoea in the study populations. In any case, the significantly lower frequency of diarrhoea supports

that early treatment of *Isospora* spp. infection can have a marked effect on the clinical implications of the infection. Similarly, Daugschies et al. (2000) observed that treatment of puppies 3 days after experimental infection with *I. ohioensis*-complex effectively suppressed haemorrhagic diarrhoea which was seen in the untreated control dogs.

The three laboratory studies demonstrated high efficacy of emodepside plus toltrazuril suspension against *I. canis* and *I. ohioensis*-complex. As it is not possible to clearly determine the stage of infection in practice, efficacy was demonstrated choosing various time points for treatment to cover a wide range from early prepatent to fully developed patent infection. Two field studies complemented the picture under natural conditions showing 99–100 % efficacy of emodepside plus toltrazuril suspension against prepatent or patent *Isospora* spp. infection based on FOC reduction in comparison to an untreated control or a reference product (Altreuther et al. 2011). The data confirmed that from an epidemiological as well as a clinical point of view it makes sense to treat at an early stage of infection to minimise oocyst shedding as well as intestinal mucosal damage. As *Isospora* spp. are primarily spread within and between litters by infected mates (Daugschies et al. 2000), it is advisable to treat all dogs within a group where infection is suspected. In contrast to coccidia infections of other species, e.g. *Isospora* suis in pigs, no exact pattern for *Isospora* spp. infection has been observed for the dog. Buehl et al. (2006) found that already 3 week old puppies had been infected and *Isospora* could still be identified in puppies shortly before weaning in the 10th week of life with a peak around the 7th week of life. Daugschies et al. (2000) reported the highest probability of oocyst excretion in puppies of 4–6 weeks of age based on evaluations conducted at several commercial dog breeders. However, also stressful situations like shipping, weaning or change in ownership are described to lead to repeated oocyst shedding and potentially clinical disease in puppies that have been exposed to *Isospora* spp. (Dubey et al. 2009; Lappin 2010) so that each treatment should be decided considering the situation of the individual puppy. If anticoccidial treatment needs to be repeated, emodepside plus toltrazuril suspension can be used if there is a concomitant need for deworming, e.g. in puppies that are treated against *Toxcara canis* according to the recommendation of the European scientific counsel companion animal parasites (ESCCAP 2010).

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Compliance statement

All of the studies reported herein were performed in compliance with current, applicable, local laws and regulations.

Disclosure statement

G. Altreuther, N. Gasda, I. Schroeder, A. Schimmel and K. J. Krieger were employed by Bayer Animal Health GmbH, Germany, and T. Settje and D. Hutchens were employed by Bayer HealthCare LLC, USA, during the conduct of the studies. Studies I and III were conducted at Bayer Animal Health GmbH, Germany. A. Joachim was an employee of the University of Veterinary Medicine Vienna, Austria, and conducted study II as CRO. All studies were sponsored by Bayer Animal Health GmbH.

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